

EDITORIAL



## The mystery of evacetrapib - why are CETP inhibitors failing?

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Cholesteryl ester transfer protein (CETP) plays a pivotal role in lipoprotein metabolism, primarily by virtue of its ability to facilitate transfer of esterified cholesterol from high-density lipoproteins (HDL) to very low-density lipoproteins (VLDL) and low-density lipoprotein (LDL) [1]. There has been considerable interest in pharmacological approaches to CETP inhibition on the basis of observations from both population studies [2] and cohorts with genetic polymorphisms [1,3,4] demonstrating low CETP activity to associate with high levels of HDL cholesterol and low rates of cardiovascular disease. A range of CETP inhibitory strategies including small molecule inhibitors, antisense oligonucleotides, and vaccination have proven to be atheroprotective in rabbit models [5–8]. Accordingly, CETP inhibition attracted considerable interest as a pharmacological approach to raising HDL cholesterol and potentially reducing cardiovascular risk.

However, multiple clinical development programs of CETP inhibitors have produced disappointing results. This has positioned CETP inhibition in the growing pile of promising pharmacological targets that have failed to convincingly reduce cardiovascular risk [9–13]. Many pundits have opined that CETP inhibitors have failed and the field should move on to alternative approaches in order to reduce the residual clinical risk observed in patients treated with established medical therapies. Careful interrogation, however, of the totality of data generated from these development programs, combined with emerging insights from genomic analyses suggests potential new avenues for the pursuit of CETP inhibitor development in clinical practice.

Torcetrapib was the first CETP inhibitor to achieve an advanced phase of clinical development. Early studies demonstrated HDL cholesterol raising by more than 60% and incremental lowering of LDL cholesterol by up to 20% on top of statin therapy [2,14]. However, a large clinical outcomes trial was terminated prematurely due to the finding of a 25% increase in the primary endpoint and 58% increase in mortality in torcetrapib treated patients [11]. Parallel vascular imaging studies demonstrated that similar lipid effects failed to favorably modulate the progression of either coronary atherosclerosis or carotid intima-medial thickness [15–17]. Subsequent analyses revealed that torcetrapib resulted in excessive adrenal production of aldosterone and cortisol and arterial wall expression of endothelin, in addition to its well-established ability to

raise blood pressure [11,18,19]. The finding that these effects occurred in rodents which do not express CETP suggested a potential off-target toxicity of the molecule [20]. While the specific mechanism underlying the clinical toxicity remains uncertain, these findings did suggest that the ability to develop a CETP inhibitor, without such toxicity, might still prove beneficial for patients with cardiovascular disease.

Dalcetrapib is a modest CETP inhibitor, raising HDL cholesterol by up to 30% and with no discernible impact on LDL cholesterol levels. Small clinical trials established no increase in vascular inflammation or presence of endothelial dysfunction with dalcetrapib, suggesting a lack of torcetrapib like toxicity [21,22]. A large clinical outcomes trial was stopped due to clinical futility [12]. A post hoc pharmacogenomic analysis of this trial revealed that patients carrying the AA rs1967309 polymorphism of adenylate cyclase 9 on chromosome 16 demonstrated less cardiovascular events in the dalcetrapib group, associated with greater increases in *ex vivo* cholesterol efflux capacity and a lack of increase in inflammatory markers, compared with dalcetrapib treated patients without this polymorphism [23]. This has led to a new cardiovascular outcomes trial performed exclusively in patients with this polymorphism [24].

Evacetrapib is a more potent CETP inhibitor, raising HDL cholesterol by more 125% and lowering LDL cholesterol by more than 30%, when administered either as monotherapy or in addition to statin therapy [25]. In a similar fashion to previous CETP inhibitors, the clinical outcomes trial was stopped prematurely for clinical futility with no evidence of event curve separation after a median treatment period of 26 months [13]. While subsequent lipid studies revealed that the HDL cholesterol increase with evacetrapib was accompanied by elevations in levels of both apolipoproteins E and C-III [26], there is currently no evidence that this has a deleterious effect at the level of the artery wall. Accordingly, the mechanism underlying the lack of clinical benefit, despite incremental LDL cholesterol lowering and raising of HDL cholesterol remained uncertain.

Anacetrapib is an additional potent CETP inhibitor, raising HDL cholesterol by more than 130% and lowering LDL cholesterol by more than 30% [27]. These findings were observed in patients with both high cardiovascular risk and also in those

with familial hypercholesterolemia [28]. An early safety study performed in more than 1600 high vascular risk patients not only demonstrated a lack of torcetrapib like toxicity, but also reported a reduction in cardiovascular events, primarily due to a lower need for coronary revascularization [27]. The largest CETP inhibitor clinical outcomes trial performed to date demonstrated a statistically significant 9% reduction in cardiovascular events in patients treated with anacetrapib for a median of 4.1 years [29]. Analysis of this trial demonstrated a direct association between non-HDL cholesterol levels and cardiovascular events. A subsequent report of longer-term follow-up, after the trial, demonstrated a greater reduction in cardiovascular events in the anacetrapib treated patients [29]. While this finding provided ultimate evidence that the administration of a CETP inhibitor could reduce cardiovascular risk, there were lingering concerns regarding adipose tissue accumulation of the drug [30] and ultimately clinical development did not proceed to the regulatory approval stage.

One additional potent CETP inhibitor, TA-8995, was evaluated in early clinical trials with evidence of more effective HDL cholesterol raising and LDL cholesterol lowering than other agents, when administered at much lower doses [31]. To date, this agent has not proceeded to the setting of a large cardiovascular outcomes trial.

For more than a decade considerable resources were focused on the potential for CETP inhibition to reduce cardiovascular risk. However, despite all of the optimism, four large outcomes trial demonstrated a combination of toxicity, clinical futility, and modest clinical benefit after prolonged treatment. The question that remains is how best to conclude the totality of data with regard to this class of pharmacological agents? Has CETP inhibition proven to be a failed strategy and should the field move on to other targets? Alternatively, does the finding of benefit with anacetrapib and potential benefit with dalcetrapib in patients with specific genetic polymorphisms leave the door open for new development programs? What ultimately are the implications for HDL raising? While some proposed that generating large, cholesterol rich, HDL particles with CETP inhibition may impair HDL functionality [32], there is currently no data to support this.

The pharmacogenomic analysis of the dalcetrapib outcomes trial demonstrates not only a clinical benefit, but also potential mechanisms underscoring this finding. However, similar analyses performed with both evacetrapib and anacetrapib have failed to replicate this finding [33,34]. This suggests that if the dalcetrapib finding is real, it reflects a molecule, rather than CETP, specific phenomenon. Ultimately, the ongoing pharmacogenomic targeted trial will determine whether this strategy works. In parallel, recent genomic studies [3,4] and the use of Mendelian randomization [35] have supported the concept that CETP inhibition continues to present a potential cardioprotective strategy [3,4,35]. This analysis suggested that the degree of CETP inhibition achieved with more potent pharmacological inhibitors should associate with a reduction in cardiovascular risk that is directly proportional to decreases in levels of apolipoprotein B. While receiving far less attention compared with HDL cholesterol raising, the concept of lowering levels of atherogenic lipoproteins with CETP inhibitors makes sense given the fundamental movement of esterified

cholesterol from HDL to VLDL and LDL in the setting of normal CETP function. In addition to reduced cholesterol content of LDL particles, kinetic studies have demonstrated an increase in the fractional catabolic rate of LDL particles, which may be enhanced in the setting of accelerated removal of triglyceride-rich LDL particles, which would be more prevalent in the setting of CETP inhibition [36]. This has been reaffirmed by observations that the more potent CETP inhibitors do reduce levels of both LDL and non-HDL cholesterol. Overall, this suggests that efforts to reduce risk via CETP inhibition might be better served by focusing on its ability to lower atherogenic lipoproteins as opposed to prior efforts that have been driven by the interest in raising HDL cholesterol.

Additional genetic analyses demonstrated that the potential benefit of less CETP is observed in the presence, but not absence, of hydroxy-methyl-glutaryl coenzyme A reductase [35]. This provides genetic evidence to suggest that the administration of CETP inhibitors might be more likely to work in the absence of statin therapy. The biochemical plausibility for this concept lies in the fact that removal of LDL from the systemic circulation with both statins and CETP inhibitors involve the LDL receptor. This introduces the potential for saturation of LDL uptake by the liver and may support greater LDL cholesterol and potentially greater event reduction with CETP inhibitors administered in the absence of statins. Whether this will lead to efficacy of CETP inhibition, or other novel lipid lowering agents, in patients with statin intolerance remains to be tested in clinical trials.

A parallel observation from these large clinical outcomes trials has been the potential impact of CETP inhibitors on the subsequent development of type 2 diabetes. In each of these studies, regardless of the ultimate impact on cardiovascular events, the administration of a CETP inhibitor was observed to associate with a lower rate of diagnosis of new cases of type 2 diabetes and improved glycemic control in those with diabetes at baseline [37,38]. While the specific mechanism underlying this observation remains uncertain, there is evidence that HDL exert favorable effects on pancreatic  $\beta$ -cell function [39,40], which may contribute to a slower rate of progression from early dysglycemia to fully established diabetes. How this observation will impact clinical practice and whether it will influence the design of any future clinical development program for CETP inhibitors is unknown at this point.

Additional approaches to CETP inhibition could take into consideration potential alternative therapeutic uses. In addition to its fundamental role in reverse cholesterol transport, HDL has been demonstrated to possess anti-inflammatory activity [41,42] that may play a role in the response to sepsis. This activity is likely to reflect a number of biological mechanisms, including direct binding of lipopolysaccharide in the setting of systemic endotoxemia [43], in addition to modulating a range of inflammatory pathways either directly or via reduction in generation of lipid peroxidation products [41]. Paraoxonase, a factor carried on HDL, has been demonstrated to possess anti-oxidant activity *in vivo* in addition to protection against cardiovascular disease [44]. This may also contribute to the anti-inflammatory potential of HDL. Additional observations that Kupfer cells lose their expression of CETP in the setting of gram-negative septicemia, with the resulting HDL playing a potential anti-endotoxin effect [43]. While

some have proposed that CETP inhibition may have potential clinical utility in the setting of sepsis, it has also been demonstrated that some of the toxicity observed with torcetrapib involved an increase in mortality attributed to sepsis [11].

In summary, increasing interest in the ability to raise HDL cholesterol levels stimulated a large amount of activity in the CETP inhibition field. Despite this optimism, clinical trials have proven disappointing, with only one study demonstrating marginal clinical benefit on relatively longer-term follow-up. More recent studies of the genetics of CETP and the potential impact of CETP inhibition on the natural history of type 2 diabetes continue to provide some hope that all will not be lost with regard to this field of agents. Whether new programs will incorporate the lessons from the past to develop novel approaches to use of CETP inhibitors remains to be determined. For now, they remain another class of tried and tested agents that have failed to reduce the substantial cardiovascular risk that many patients harbor despite the use of established therapies.

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## References

Papers of special note have been highlighted as either of interest (\*) or of considerable interest (\*\*\*) to readers.

- Barter PJ, Chapman MJ, Hennekens CH, et al. Cholesteryl ester transfer protein. A novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2003;23(2):160–167.
- Brousseau ME, Schaefer EJ, Wolfe ML, et al. Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. *N Engl J Med.* 2004 Apr 8;350(15):1505–1515.
- Thompson A, Di Angelantonio E, Sarwar N, et al. 2008 Jun 18. Association of cholesteryl ester transfer protein genotypes with cetp mass and activity, lipid levels, and coronary risk. *Jama* 299(23):2777–2788.
- Johannsen TH, Frikke-Schmidt R, Schou J, et al. Genetic inhibition of CETP, ischemic vascular disease and mortality, and possible adverse effects. *J Am Coll Cardiol.* 2012 Nov 13;60(20):2041–2048.
- Rittershaus CW, Miller DP, Thomas LJ, et al. Vaccine-induced antibodies inhibit CETP activity in vivo and reduce aortic lesions in a rabbit model of atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2000 Sep;20(9):2106–2112.
- Sugano M, Makino N, Sawada S, et al. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. *J Biol Chem.* 1998 Feb 27;273(9):5033–5036.
- Okamoto H, Yonemori F, Wakitani K, et al. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature.* 2000 Jul 13;406(6792):203–207.
- Morehouse LA, Sugarman ED, Bourassa PA, et al. Inhibition of CETP activity by torcetrapib reduces susceptibility to diet-induced atherosclerosis in New Zealand White rabbits. *J Lipid Res.* 2007 Jun;48(6):1263–1272.
- HE B. ACP Journal Club: adding niacin plus laropiprant to statins did not reduce vascular events and increased serious adverse events. *Ann Intern Med.* 2014 Nov 18;161(10):JC8.
- HTC G, MJ L, Haynes R, et al. Effects of extended-release niacin with laropiprant in high-risk patients. *N Engl J Med.* 2014 Jul 17;371(3):203–212.
- Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med.* 2007 Nov 22;357(21):2109–2122.
- Schwartz GG, Olsson AG, Abt M, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med.* 2012 Nov 29;367(22):2089–2099.
- Lincoff AM, Nicholls SJ, Riesmeyer JS, et al. Evacetrapib and cardiovascular outcomes in high-risk vascular disease. *N Engl J Med.* 2017 May 18;376(20):1933–1942.
- Clark RW, Sutfin TA, Ruggeri RB, et al. Raising high-density lipoprotein in humans through inhibition of cholesteryl ester transfer protein: an initial multidose study of torcetrapib. *Arterioscler Thromb Vasc Biol.* 2004 Mar;24(3):490–497.
- Nissen SE, Tardif JC, Nicholls SJ, et al. Effect of torcetrapib on the progression of coronary atherosclerosis. *N Engl J Med.* 2007 Mar 29;356(13):1304–1316.
- Bots ML, Visseren FL, Evans GW, et al. Torcetrapib and carotid intima-media thickness in mixed dyslipidaemia (RADIANCE 2 study): a randomised, double-blind trial. *Lancet.* 2007 Jul 14;370(9582):153–160.
- Kastelein JJ, van Leuven SI, Burgess L, et al. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. *N Engl J Med.* 2007 Apr 19;356(16):1620–1630.
- Barter P. Lessons learned from the investigation of lipid level management to understand its impact in atherosclerotic events (ILLUMINATE) trial. *Am J Cardiol.* 2009 Nov 16;104(10 Suppl):10E–5E.
- Vergeer M, Stroes ES. The pharmacology and off-target effects of some cholesterol ester transfer protein inhibitors. *Am J Cardiol.* 2009 Nov 16;104(10 Suppl):32E–8E.
- Forrest MJ, Bloomfield D, Briscoe RJ, et al. Torcetrapib-induced blood pressure elevation is independent of CETP inhibition and is accompanied by increased circulating levels of aldosterone. *Br J Pharmacol.* 2008 Aug;154(7):1465–1473.
- Luscher TF, Taddei S, Kaski JC, et al. Vascular effects and safety of dalcetrapib in patients with or at risk of coronary heart disease: the dal-VESSEL randomized clinical trial. *Eur Heart J.* 2012 Apr;33(7):857–865.
- Fayad ZA, Mani V, Woodward M, et al. Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging (dal-PLAQUE): a randomised clinical trial. *Lancet.* 2011 Oct 29;378(9802):1547–1559.
- Tardif JC, Rheume E, Lemieux Perreault LP, et al. Pharmacogenomic determinants of the cardiovascular effects of dalcetrapib. *Circ Cardiovasc Genet.* 2015 Jan 11;8(2):372–382.
- \*\* Pharmacogenomic analysis revealing potential benefits of dalcetrapib.**
- Tardif J-C, Dube M-P, Pfeiffer MA, et al. Study design of Dal-GenE, a pharmacogenetic trial targeting reduction of cardiovascular events with dalcetrapib. *Am Heart J.* 2020;222:157–165.
- Nicholls SJ, Brewer HB, Kastelein JJP, et al. Effects of the CETP inhibitor evacetrapib administered as monotherapy or in combination with

- statins on HDL and LDL cholesterol: a randomized controlled trial. *JAMA*. 2011 Nov 16;306(19):2099–2109.
26. Nicholls SJ, Ray KK, Ballantyne CM, et al. Comparative effects of cholesteryl ester transfer protein inhibition, statin or ezetimibe on lipid factors: the ACCENTUATE trial. *Atherosclerosis*. 2017 Jun;261:12–18.
  27. Cannon CP, Shah S, Dansky HM, et al. Safety of anacetrapib in patients with or at high risk for coronary heart disease. *N Engl J Med*. 2010 Dec 16;363(25):2406–2415.
  28. Kastelein JJ, Besseling J, Shah S, et al. Anacetrapib as lipid-modifying therapy in patients with heterozygous familial hypercholesterolaemia (REALIZE): a randomised, double-blind, placebo-controlled, phase 3 study. *Lancet*. 2015 May 30;385(9983):2153–2161.
  29. HTRC. Effects of anacetrapib in patients with atherosclerotic vascular disease. *N Engl J Med*. 2017 Sep 28;377(13):1217–1227.
    - **Clinical benefit of anacetrapib.**
  30. Gotto AM Jr., Kher U, Chatterjee MS, et al. Lipids, safety parameters, and drug concentrations after an additional 2 years of treatment with anacetrapib in the DEFINE study. *J Cardiovasc Pharmacol Ther*. 2014 Nov;19(6):543–549. .
  31. Hovingh GK, Kastelein JJ, van Deventer SJ, et al. Cholesterol ester transfer protein inhibition by TA-8995 in patients with mild dyslipidaemia (TULIP): a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet*. 2015 Aug 1;386(9992):452–460.
  32. Barter PJ. CETP and atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2000;20(9):2029–2031.
  33. Hopewell JC, Ibrahim M, Hill M, et al. Impact of ADCY9 genotype on response to anacetrapib. *Circulation*. 2019 Jul 23;140(11):891–898.
  34. Nissen SE, Pillai SG, Nicholls SJ, et al. ADCY9 genetic variants and cardiovascular outcomes with evacetrapib in patients with high-risk vascular disease: a nested case-control study. *JAMA Cardiol*. 2018 May 1;3(5):401–408.
  35. Ference BA, Kastelein JJP, Ginsberg HN, et al. Association of genetic variants related to CETP inhibitors and statins with lipoprotein levels and cardiovascular risk. *JAMA*. 2017 Sep 12;318(10):947–956. .
    - **Mendelian randomization data revealing potential benefits of CETP deficiency.**
  36. Millar JS, Reyes-Soffer G, Jumes P, et al. Anacetrapib lowers LDL by increasing ApoB clearance in mildly hypercholesterolemic subjects. *J Clin Invest*. 2015 Jun;125(6):2510–2522. .
  37. Masson W, Lobo M, Siniawski D, et al. Therapy with cholesteryl ester transfer protein (CETP) inhibitors and diabetes risk. *Diabetes Metab*. 2018 Feb 20;44(6):508–513.
  38. Barter PJ, Rye KA, Tardif JC, et al. Effect of torcetrapib on glucose, insulin, and hemoglobin A1c in subjects in the investigation of lipid level management to understand its impact in atherosclerotic events (ILLUMINATE) trial. *Circulation*. 2011 Aug 2;124(5):555–562. .
    - **Potential benefit of CETP inhibition on glycemic control in diabetes.**
  39. Barter PJ, Cochran BJ, Rye KA. CETP inhibition, statins and diabetes. *Atherosclerosis*. 2018 Sep;26(278):143–146.
  40. von Eckardstein A, Widmann C. High-density lipoprotein, beta cells, and diabetes. *Cardiovasc Res*. 2014 Aug 1;103(3):384–394.
  41. Barter PJ, Nicholls S, Rye KA, et al. Antiinflammatory properties of HDL. *Circ Res*. 2004 Oct 15;95(8):764–772.
  42. SJ N, GJ D, Cutri B, et al. Reconstituted high density lipoproteins inhibit the acute pro-oxidant and proinflammatory vascular changes induced by a periarterial collar in normocholesterolemic rabbits. *Circulation*. 2005;111(12):1543–1550. .
  43. Blauw LL, Wang Y, Willems van Dijk K, et al. A novel role for CETP as immunological gatekeeper: raising HDL to cure sepsis? *Trends Endocrinol Metab*. 2020 Feb 4. DOI:10.1016/j.tem.2020.01.003.
  44. Bhattacharyya T, Nicholls SJ, Topol EJ, et al. Relationship of para-oxonase 1 (pon1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *Jama*. 2008 Mar 19;299(11):1265–1276.